# HOMOGENEOUS GENETIC STRUCTURE OF *MEGANYCTIPHANES NORVEGICA* (EUPHAUSIACEA) IN THE NORTH-EAST ATLANTIC OCEAN, AS INTERPRETED FROM ALLOZYMIC VARIATION

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SARSIA

SUNDT, ROLF C. & SVEIN E. FEVOLDEN 1996 07 01. Homogeneous genetic structure of *Meganyctiphanes norvegica* (Euphausiacea) in the north-east Atlantic Ocean, as interpreted from allozymic variation. — *Sarsia* 81:155-159. Bergen. ISSN 0036-4827.

In order to elucidate the possible existence of genetically separated stocks of *Meganyctiphanes norvegica* in the north-east Atlantic Ocean, a population genetic analysis of the species was commenced. In particular we wanted to investigate how possible genetic cohesiveness between samples corresponds to the physical oceanographic structure in the region. An electrophoretic analysis of allele distributions at five enzyme loci identified in previous studies as being polymorphic was screened for variation between samples. 1 043 individuals from eight locations were studied and with the genetic resolution attained with allozymes on the loci studied, samples appear to represent a single panmictic population.

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KEYWORDS: Euphausiacea; *Meganyctiphanes norvegica;* north-east Atlantic; allozymes; genetic structure.

## INTRODUCTION

*Meganyctiphanes norvegica* (M. Sars) is a relative large (total length, 40 mm) epipelagic euphausiid found in the North Atlantic and in the Mediterranean (EINARSSON 1945; MAUCHLINE & FISHER 1967,1969). It has two population centres in the North Atlantic, one on the American continental shelf off Nova Scotia and one in the Norwegian Sea (LINDLEY 1982). In the Norwegian Sea, it is one of the most abundant zooplankters and accounts for both the highest biomass and the highest production among the euphausiids in the region (LINDLEY 1982). *M. norvegica* is also a common zooplankter in Norwegian coastal waters and fjords (JÖRGENSEN & MATTHEWS 1975; FALK-PETTERSEN & HOPKINS 1981; KAARTVEDT & SVENDSEN 1990). In the north-east Atlantic it has been found as far as 80° N, but is less common in the Barents Sea and in Greenland waters except where there is a strong Atlantic water influence (DUNBAR 1964; MAUCHLINE & FISHER 1967; DALPADADO & SKJOLDAL 1991).

Various characteristics of the life history of *M. norvegica*, like spawning behaviour and growth, have led to the suggestion that reproductively isolated stocks may exist. Spawning begins earlier in the year in southern than in northern areas; i.e. in early January in the Mediterranean, in February in the English Channel and in early summer in Norwegian fjords and in the Gulf of St. Lawrence (LEBOUR 1924; EINARSSON 1945; BERKES 1976). In the Norwegian Sea spawning is most vigorous on the edges of the coastal banks (EINARSSON 1945), but it remains to be proven whether those areas are inhabited by self-maintaining populations. Differences in growth and different developmental pathways in the furciliae from different parts of the species' distributional range are also reported (EINARSSON 1945; CASANOVA 1977, quoted by MAUCHLINE 1980), but such characteristics have not been proven to be genetically controlled; they could just as well have been induced by ecological factors. Notwithstanding, off the British Isles local spawning centres with distinct size frequencies have been described (MAUCHLINE 1959), but again no proof of genetically structured populations in those areas has been given (ANDERSON 1982; isozyme techniques). Nor have the suggested distinct populations of *M. norvegica* off the American coast, in Gulf of Maine and in Bay of Fundy (KULKA & ISLES 1975; KULKA & al. 1982), been established as genetically distinct stocks.

Most population genetic investigations conducted on euphausiids from the Southern Ocean conclude that there is a high level of genetic homogeneity between sampling areas. This includes the species *Euphausia superba* (FEVOLDEN & AYALA, 1981; FEVOLDEN 1986; KUHL & SCHNEPPENHEIM 1986; MCDONALD & al. 1986; FEVOLDEN & SCHNEPPENHEIM 1988, 1989) and *E. crystallorophias* (KUHL & SCHNEPPENHEIM 1986). It has been suggested that the

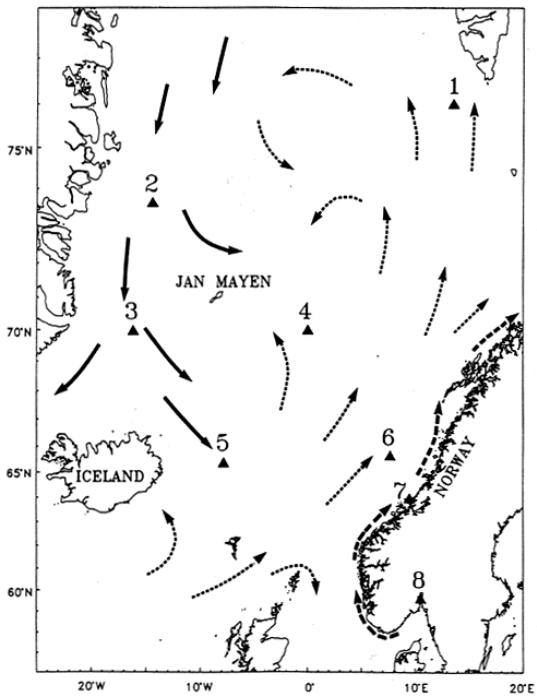


Fig. 1. Sampling localities of *Meganyctiphanes norvegica* in the north-east Atlantic. Major surface currents are indicated. ·····> = Atlantic currents, —> = polar currents, - - -> = Norwegian Coastal Current.

lack of genetic heterogeneity between Southern Ocean areas is the 'mixing nature' of the hydrographical mechanisms of the East Wind Drift and the Antarctic Circumpolar Current (Fevolden & Schneppenheim 1989). On the other hand, investigations on the species *E. krohnii* and *Nematoscelis megalops*, from north-west Atlantic slope waters, show some evidence of genetic structuring (Bucklin & Wiebe 1986). The oceanographic features in that area may be considered less conducive to dispersal of planktonic species than the Southern Ocean.

The current flow in the north-east Atlantic is dominated by Atlantic water in the south and polar water in the north (Blindheim 1989). Eddies or oceanic gyres in and between the Norwegian and Greenland Seas could prevent movement of planktonic animals between different areas, and therefore it is possible that genetically different populations of *M. norvegica* could have evolved. It is, as yet, an open question whether *M. norvegica* performs an active spawning migration to restricted spawning grounds. In order to elucidate the possible existence of genetically separated stocks of *M. norvegica* in the north-east Atlantic, a population genetic analysis of the species was commenced. In particular we wanted to investigate how possible genetic cohesiveness between samples correspond to the physical oceanographic structure in the north-east Atlantic.

## MATERIALS AND METHODS

Samples were collected during cruises in 1993, 1994, and 1995 using ring trawl, (Munk 1988) and pelagic fish trawl (Valdemarsen & Misund 1995). Samples 1, 4, and 6 were collected in Atlantic water masses, samples 2, 3, and 5 were from polar water masses and samples 7 and 8 were collected in the Norwegian coastal current. The sample localities and capture dates are listed in Table 1 (sample localities are also shown in Fig. 1). Live krill were immediately frozen individually at -70° C and stored for up to six months before analyzed. To avoid confusion with other species only adult individuals were analyzed. Samples for electrophoresis were prepared by sonicating muscle tissue in equal amounts of distilled water. In order to avoid sample contamination from digestive enzymes and gut contents only muscle tissue was analyzed.

Pieces of filter-paper (10 mm x 1.5 mm) were soaked in the cell lysates and applied to the gels. Horizontal gel electrophoresis using a histidine/citrate buffer-system with pH 7.0 (Ward & Beardmore 1977) was performed. This gel buffer allowed all the assayed loci to be resolved in the same gel. Only enzymes catalyzing reactions involving internally derived substances were used as possible genetic markers.

Table 1. Sample localities and capture dates of *Meganyctiphanes norvegica* in the north-east Atlantic.

| Sample | | Pos. | Date |
|---|---|---|---|
| 1 | Storfjordrenna | 76°01' N - 13°33' E | 5 Aug 94 |
| 2 | Vesterisbanken | 73°37' N - 14°24' W | 2 Aug 94 |
| 3 | North of Iceland | 70°00' N - 16°08' W | 23 Jul 94 |
| 4 | Central Norwegian Sea | 70°01' N - 01°39' E | 23 Nov 93 |
| 5 | East of Iceland | 65°23' N - 07°45' W | 12 Jul 93 |
| 6 | Norwegian shelf | 65°42' N - 07°47' E | 7 Jun 94 |
| 7 | Frohavet | 63°59' N - 09°29' E | 14 Dec 93 |
| 8 | Oslofjorden | 59°30' N - 10°30' E | 8 May 95 |

Starch gels of 12.5 % and 8 mm thick were run at 230 V until a control marker of bromphenol blue migrated out of the gel. Each gel was sliced into four sheets 1 mm thick and stained for lactate dehydrogenase (LDH, E.C. 1.1.1.27), mannose-phosphate isomerase (MPI, E.C. 5.3.1.8.), glucosephosphate isomerase (GPI, E.C. 5.3.1.9.), and phosphoglucomutase (PGM, E.C. 2.7.5.1.) according to Fevolden & Ayala (1981) and Fevolden (1982) (MPI). Polymorphic loci have previously been described by Anderson (1982) and Fevolden (1982). Stained gels were preserved for future analyses by drying on filter-paper.

The different alleles are assigned figures where 100 designates the most common allele. The designations of the remaining alleles indicates their percentage mobility relative to the 100 allele for the gel buffer employed here. Allelic correspondence between the present study and Anderson (1982) and Fevolden (1982) has not been established, and therefore the allelic designations might be different in the studies. Statistical analysis of the electrophoretic data was facilitated using the computer program BIOSYS-1 (Swofford & Selander 1981).

## RESULTS

Allelic frequencies at the five polymorphic loci are given in Table 2. With the exception of PGM in sample 3, no significant deviations from Hardy-Weinberg distributions of genotypes were observed ($p \geq 0.157$; $p = 0.041$ for the deviant sample set). Contingency chi-square analyses of the allele frequencies (Table 3), gave no evidence of a genetic differentiation among the various sample groups of *M. norvegica*. This conclusion is also evident from estimates of the genetic identity coefficient (Nei 1972) ($I > 0.99$ for all pairwise comparisons of samples).

## DISCUSSION

As a preliminary approach to resolve the genetic population structure of *M. norvegica* in the north-east Atlantic, isozyme analyses were applied. However, the data pre-

Table 2.*Meganyctiphanes norvegica.* Allele frequencies in eight samples from the north-east Atlantic. Locations as in Table 1, enzyme abbreviations see text. (N = sample size).

| Locus | Allele | Collecting stations | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| *Ldh -1* | *70* | 0.003 | 0.014 | 0 | 0.005 | 0 | 0 | 0 | 0.003 |
| | *90* | 0 | 0.007 | 0.003 | 0 | 0.005 | 0 | 0 | 0.008 |
| | *100* | 0.997 | 0.972 | 0.990 | 0.984 | 0.990 | 0.997 | 0.995 | 0.983 |
| | *105* | 0 | 0.007 | 0.007 | 0.011 | 0.005 | 0.003 | 0.005 | 0.006 |
| (N) | | (144) | (144) | (144) | (93) | (96) | (144) | (96) | (182) |
| *Ldh - 2* | *90* | 0.042 | 0.045 | 0.049 | 0.022 | 0.031 | 0.045 | 0.036 | 0.023 |
| | *100* | 0.951 | 0.948 | 0.948 | 0.978 | 0.969 | 0.948 | 0.958 | 0.974 |
| | *110* | 0.007 | 0.007 | 0.007 | 0 | 0 | 0.007 | 0.005 | 0.003 |
| (N) | | (144) | (144) | (144) | (93) | (96) | (144) | (96) | (182) |
| *Mpi* | *75* | 0.007 | 0 | 0.003 | 0 | 0 | 0 | 0 | 0 |
| | *90* | 0.076 | 0.092 | 0.097 | 0.061 | 0.068 | 0.058 | 0.069 | 0.052 |
| | *100* | 0.590 | 0.612 | 0.583 | 0.591 | 0.573 | 0.622 | 0.586 | 0.627 |
| | *105* | 0.306 | 0.285 | 0.302 | 0.335 | 0.359 | 0.320 | 0.345 | 0.315 |
| | *110* | 0.007 | 0.012 | 0.014 | 0.012 | 0 | 0 | 0 | 0.012 |
| (N) | | (144) | (131) | (144) | (82) | (96) | (139) | (87) | (165) |
| *Gpi* | *75* | 0.003 | 0.007 | 0 | 0.005 | 0 | 0.003 | 0 | 0.006 |
| | *80* | 0.024 | 0.017 | 0.017 | 0.022 | 0.016 | 0.031 | 0.031 | 0.017 |
| | *100* | 0.951 | 0.958 | 0.958 | 0.962 | 0.952 | 0.955 | 0.953 | 0.941 |
| | *120* | 0.021 | 0.017 | 0.024 | 0.011 | 0.032 | 0.010 | 0.016 | 0.036 |
| (N) | | (144) | (144) | (144) | (93) | (93) | (144) | (96) | (182) |
| *Pgm* | *85* | 0.003 | 0 | 0.007 | 0 | 0 | 0 | 0.010 | 0 |
| | *90* | 0.042 | 0.035 | 0.035 | 0.038 | 0.047 | 0.038 | 0.036 | 0.060 |
| | *100* | 0.941 | 0.948 | 0.951 | 0.946 | 0.930 | 0.948 | 0.953 | 0.934 |
| | *105* | 0.010 | 0.014 | 0.007 | 0.011 | 0.023 | 0.014 | 0 | 0.006 |
| | *115* | 0.003 | 0.003 | 0 | 0.005 | 0 | 0 | 0 | 0 |
| (N) | | (144) | (144) | (144) | (93) | (86) | (144) | (96) | (182) |

sented herein, give no evidence to suggest a genetic differentiation over the geographic area sampled. When the present sample sets are compared with previous data from the Oslofjord (Fevolden 1982), striking similarities are seen at all loci. PGM was reported to be monomorphic in that study, but this discrepancy arises from different electrophoretic mobility under the described running conditions in the two different studies. One test for deviation from Hardy-Weinberg distributions of genotypes was significant. This is probably a Type 1 error and can be expected by chance.

Physical barriers to dispersal, e.g. hydrographic structuring, can cause genetic structuring. A combination of variant current flows and biological factors like aggregative behaviour may lead to genetic structuring of previously homogeneous populations - provided, of course, the time-span since segregation is sufficiently long. Populations could have adapted to large- and mesoscale oceanographic cells in the present current pattern. Based on expected gene flow caused by advection, it would have been likely with more genetic homogeneity within each of the dominating water masses (Atlantic and polar) than it would be between the different water masses. It is known that climatic changes may alter the direction of major ocean currents. However, possible changes that have taken place in the North Atlantic since the last glaciation may be too recent for any genetic differentiation to evolve. In Norwegian fjords estimates of advective rates suggest that exchange with coastal waters is more important for the standing stock than local growth within the fjords (Kaartvedt & Svendsen 1990). A replacement like this will cause geneflow and thereby counteract the evolution of genetic distinct populations in the fjords. In Balsfjorden in northern Norway, which is a krill-rich area, Falk-Pettersen & Hopkins (1981) did not find eggs and larvae of *M. norvegica*, indicating that recruitment occurs from outside the fjord.

Adult individuals of *M. norvegica* show markedly heterogeneous dispersal patterns (Kulka & al. 1982; Nicol 1986). They seem to be able to counteract advection and stay in ecologically favourable water masses by active swimming and by exploiting different current velocities and directions in different depths. Eggs and larval stages, which inhabit the uppermost water layers do not, however, have this ability, making interregional mixing of early stages possible.

As mentioned above the distribution of *M. norvegica* shows two population centres in the North Atlantic, one on the American continental shelf and one in the Norwegian Sea (Lindley 1982). It is likely that the Gulf Stream to some extent facilitates gene flow between the two regions. Based on sequence variation for the 16s mitochondrial gene, Bucklin & al. (1996) concluded that gene flow in the copepod *Calanus finmarchicus* across the Atlantic is considerable. This copepod is much smaller than *M. norvegica*, and passive horizontal flux is likely to be more extensive. Populations of *M. norvegica* seem to be morphologically indistinguishable from each other except for certain morphological deviations found in the Mediterranean stock (Casanova 1977, quoted by Mauchline 1980). It has not been documented, however, that this population has been isolated over a longer period of time than other populations (Anderson 1982).

Homogeneity in the genes examined in this study does not necessarily infer interbreeding. Karl & Avise (1992) showed that selection works on protein electrophoretic characters that balances allozyme frequencies in American oysters. Such balancing selection could also be an alternative for the observed homogeneity in the present study. Due to the large population size of most krill species, evolution of genetic differences following reproductive isolation would be expected to be a very slow process (the relevance to *Euphausia superba* is discussed by Fevolden & Schneppenheim 1989). Theoretical considerations suggest that nearly complete demographic isolation over long periods of time may be required to produce detectable differences in allele frequencies at neutral loci. Examples exist where different sets of markers (e.g. allozymes and mtDNA) reveal different levels and patterns of geographic variation (Mitton 1994).

Table 3.*Meganyctiphanes norvegica*. Cotingency chi-square analysis at all five polymorphic loci investigated.

| Loci | No. Alleles | chi-square | D.F. | P |
|---|---|---|---|---|
| *Ldh-1* | 4 | 21.414 | 18 | 0.259 |
| *Ldh-2* | 3 | 5.789 | 12 | 0.926 |
| *Pgi* | 4 | 10.886 | 18 | 0.899 |
| *Pgm* | 4 | 15.792 | 18 | 0.895 |
| *Mpi* | 5 | 23.115 | 24 | 0.513 |
| Totals | | 76.995 | 96 | 0.923 |

ACKNOWLEDGEMENTS

This work is supported by founding from the Norwegian Council of Fisheries Research (NFFR). We thank Rita Amundsen for collection of the Oslofjord sample, and two anonymous referees for helpful comments on the manuscript.

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*Accepted 25 April 1996.*